

# Supporting Information

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## SI Materials and Methods

### Calculation of Neutral Substitution Rates in Mammalian Lineages Colonized by SPIN.

Neutral substitution rates have not been published for each of the species where *SPIN* elements are found. Therefore, we devised a method that uses alignments of ancestral DNA transposons, which are assumed to have evolved neutrally since their insertion (1, 2), as a proxy to derive the neutral substitution rate in the species lineages invaded by *SPIN*. For each target species, we identified >400 individual insertions (range = 404–1,469) from 12 different DNA transposon families present at orthologous loci in human by using a program called OrthoBlast (J.K.P. and C.F., unpublished data). Orthologous TE copies were aligned pairwise and to their ancestral consensus sequence (published in Repbase (3)) and gaps were removed to produce a 3-way alignment (target species/human/consensus). All alignments were concatenated to derive an overall substitution rate. For the opossum, we used 846 DNA transposons from six different families present at orthologous loci in opossum and wallaby. The overall percent divergence of all of the orthologous TE copies was calculated with the Kimura 2-parameter correction (4). A neutral substitution rate was then inferred by dividing the percent divergence by the estimated time elapsed since the split of the two compared species. The divergence times that were used to infer neutral substitution rates were derived from a survey of the most current literature (5–13). These divergence times, along with the calculated rates in substitutions/myr, are: Human–tenrec, 104 myr ( $2.9173 \times 10^{-9}$ ); human–bat, 94 myr ( $2.6920 \times 10^{-9}$ ); human–rodents, 89 myr ( $3.5411 \times 10^{-9}$ ); human–bushbaby, 80 myr ( $2.9590 \times 10^{-9}$ ); and opossum–wallaby, 76 myr ( $3.2113 \times 10^{-9}$ ).

We note that our estimate of the neutral substitution rate for the mouse lineage is significantly lower than the one published previously ( $4.5 \times 10^{-9}$  substitutions/myr) (1). However, this discrepancy can be largely explained by the fact that the previous study used a divergence time between rodents and humans of 75

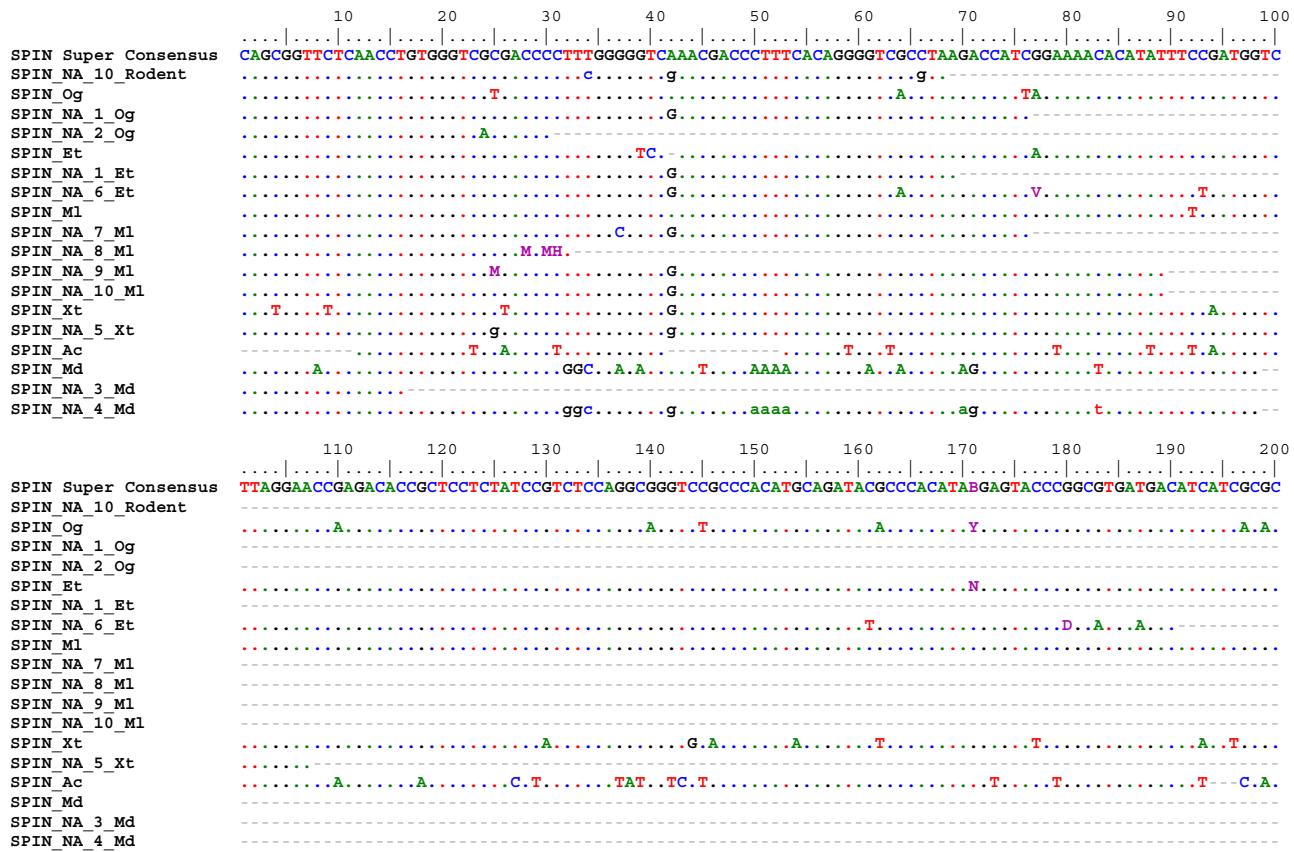
myr, whereas the most recent literature (5–13) points to a deeper divergence date (we used 89 mya based on a consensus of the literature). Because it was not possible to find a large number of orthologous repeats present either in frog or lizard and another species with known divergence time, we used a neutral substitution rate previously published in frogs ( $1.2270 \times 10^{-9}$ ) (14) to infer the age of *SPIN* elements found in *Xenopus*, but were unable to infer the age of *SPIN* in lizard.

**PCR and Sequencing of Nonautonomous SPIN Elements.** The presence of nonautonomous *SPIN* elements was verified by PCR/sequencing in all of the species where they were identified computationally (or in a close relative): Bushbaby (*Otolemur garnettii*), Ridley's bat (*Myotis ridleyi*), common tenrec (*Tenrec ecaudatus*, SMG-15088; S. M. Goodman's collection), mouse (*Mus musculus*), anole lizard (*Anolis carolinensis*), Western clawed frog (*Xenopus tropicalis*), and opossum (*Monodelphis domestica*). Two species where *SPIN* elements were not found computationally were added as negative controls: Human (*Homo sapiens*) and the Jamaican fruit bat (*Artibeus jamaicensis*).

Nonautonomous *SPIN* elements were amplified in all species but opossum by using the following PCR primers: NA-F 5'CGA ACG ACC CTT TCA CAG G (position 41–59 of the super consensus, (supporting information (SI) Fig. S1) and NA-R 5'CAG TTC CTC ATG TTG TGG TGA C (position 2878–2899). The primers used for the opossum are: NA-Fmdo 5'GGT CGC CTA AAG CCA TCG (position 60–77) and NA-Rmdo 5'GGT CGC CTA AAG CCA TCG. PCR was carried out with an initial denaturation step of 2 min at 94°C followed by 30 cycles of 1 min at 94°C, 30 s at 53°C, and 30 s at 72°C. PCR mix was: Buffer (5×), 5 μl; MgCl<sub>2</sub> (25 mM), 2 μl; dNTP (10 mM), 0.5 μl; NA-F (10 μM), 1 μl; NA-R (10 μM), 1 μl; Taq (GoTaq, Promega), 1.25 U; DNA, 30 ng; and H<sub>2</sub>O, ≤25 μl.

PCR products were cloned into the pCR2.1-TOPO cloning vector (Invitrogen) and one randomly selected clone of each species was sequenced.

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5. Janecka JE, et al. (2007) Molecular and genomic data identify the closest living relative of primates. *Science* 318:792–794.
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7. Poux C, Chevret P, Huchon D, de Jong WW, Douzery EJ (2006) Arrival and diversification of caviomorph rodents and platyrhine primates in South America. *Syst Biol* 55:228–244.
8. Poux C, et al. (2005) Asynchronous colonization of Madagascar by the four endemic clades of primates, tenrecs, carnivores, and rodents as inferred from nuclear genes. *Syst Biol* 54:719–730.
9. Delsuc F, Vizcaino SF, Douzery EJ (2004) Influence of tertiary paleoenvironmental changes on the diversification of South American mammals: A relaxed molecular clock study within xenarthrans. *BMC Evol Biol* 4:11.
10. Steiper ME, Young NM (2006) Primate molecular divergence dates. *Mol Phylogenet Evol* 41:384–394.
11. Bininda-Emonds OR, et al. (2007) The delayed rise of present-day mammals. *Nature* 446:507–512.
12. Springer MS, Murphy WJ, Eizirik E, O'Brien SJ (2003) Placental mammal diversification and the cretaceous-tertiary boundary. *Proc Natl Acad Sci USA* 100:1056–1061.
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14. Crawford AJ (2003) Relative rates of nucleotide substitution in frogs. *J Mol Evol* 57:636–641.



**Fig. S1.** Multiple alignment of *SPIN* transposons and their derived MITEs in each species. All sequences are consensus sequences, except *SPIN\_Xt* and *SPIN\_Ac*, which are individual *SPIN* copies. MITEs are denoted by "NA" (for nonautonomous). The alignment shows that the MITE families from different species have distinct deletion breakpoints with their cognate full-length *SPIN* transposon, indicating that each originated independently from a distinct deletion derivative. Sequences used are included in FASTA format following the alignment. Species abbreviations: Rodent = mouse/rat, Og = *Otolemur garnettii* (bushbaby), Et = *Echinops telfairi* (tenrec), Ml = *Myotis lucifugus* (bat), Xt = *Xenopus tropicalis* (frog), Ac = *Anolis carolinensis* (lizard), Md = *Monodelphis domestica* (opossum).

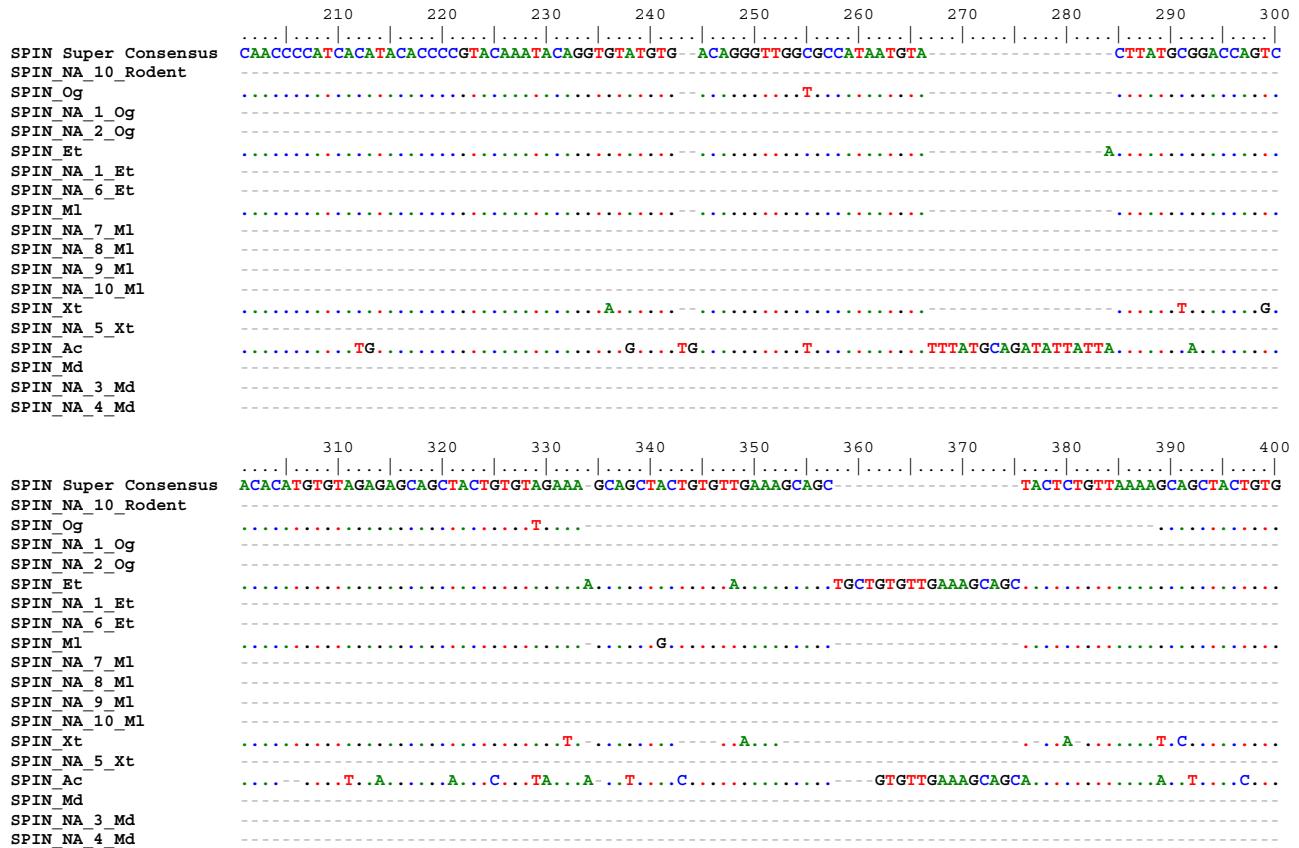


Fig. S1. (continued)

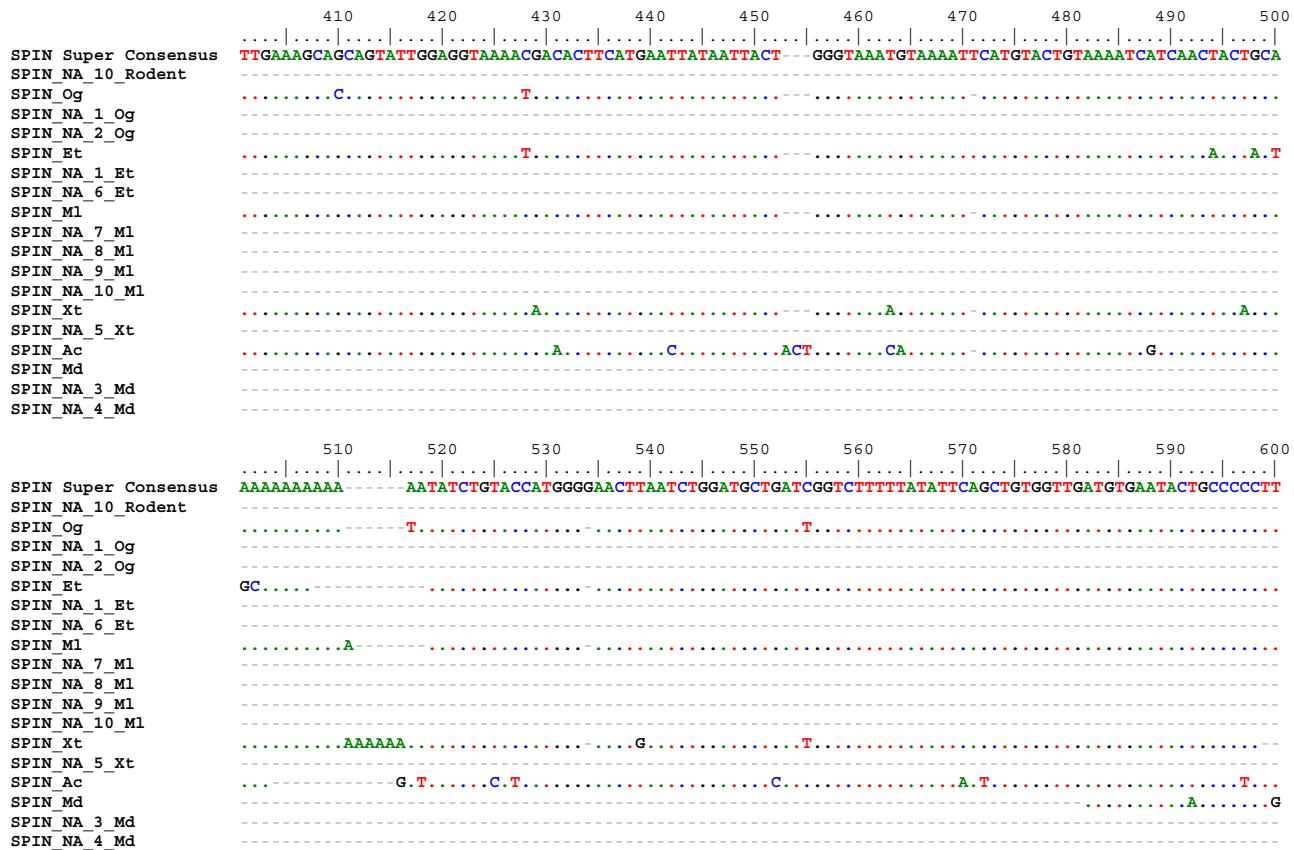


Fig. S1. (continued)

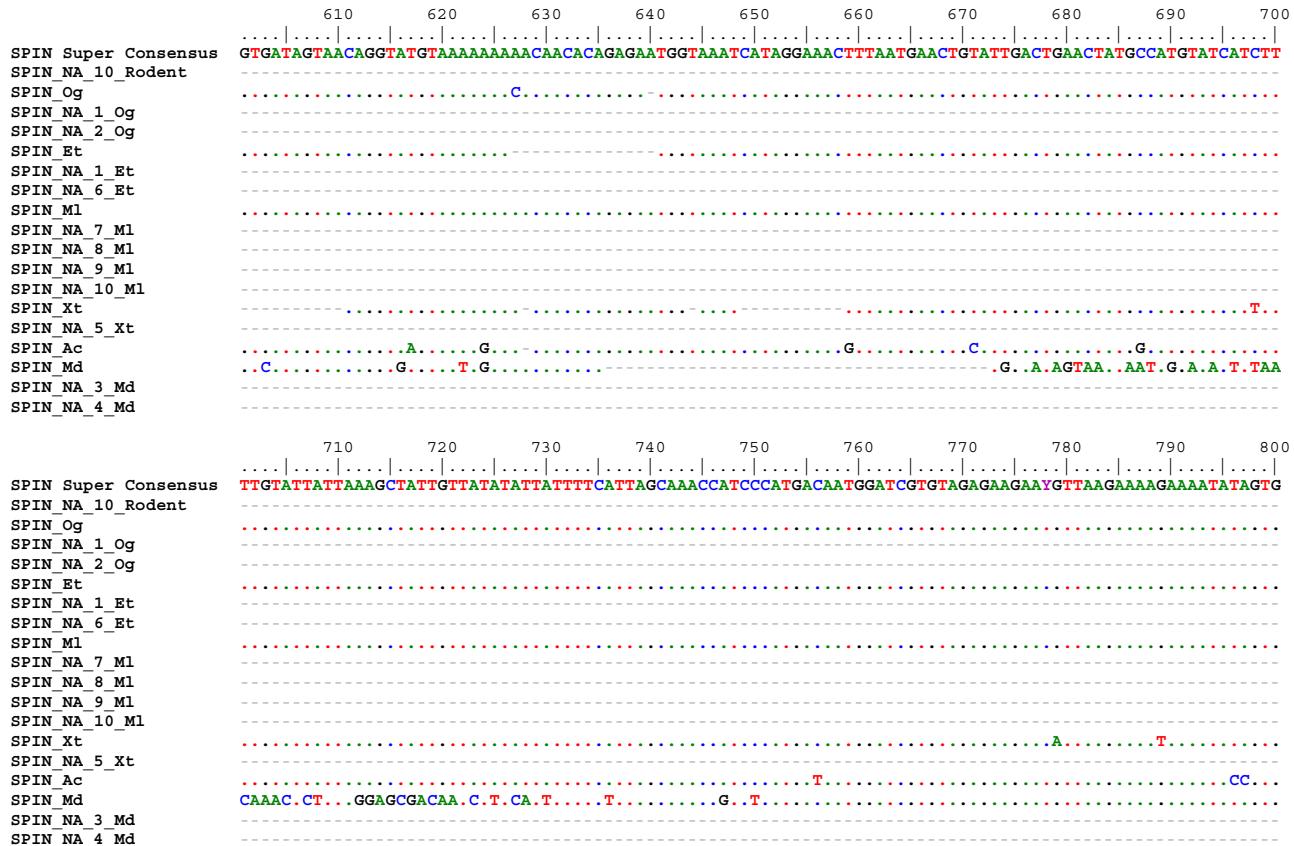


Fig. S1. (continued)

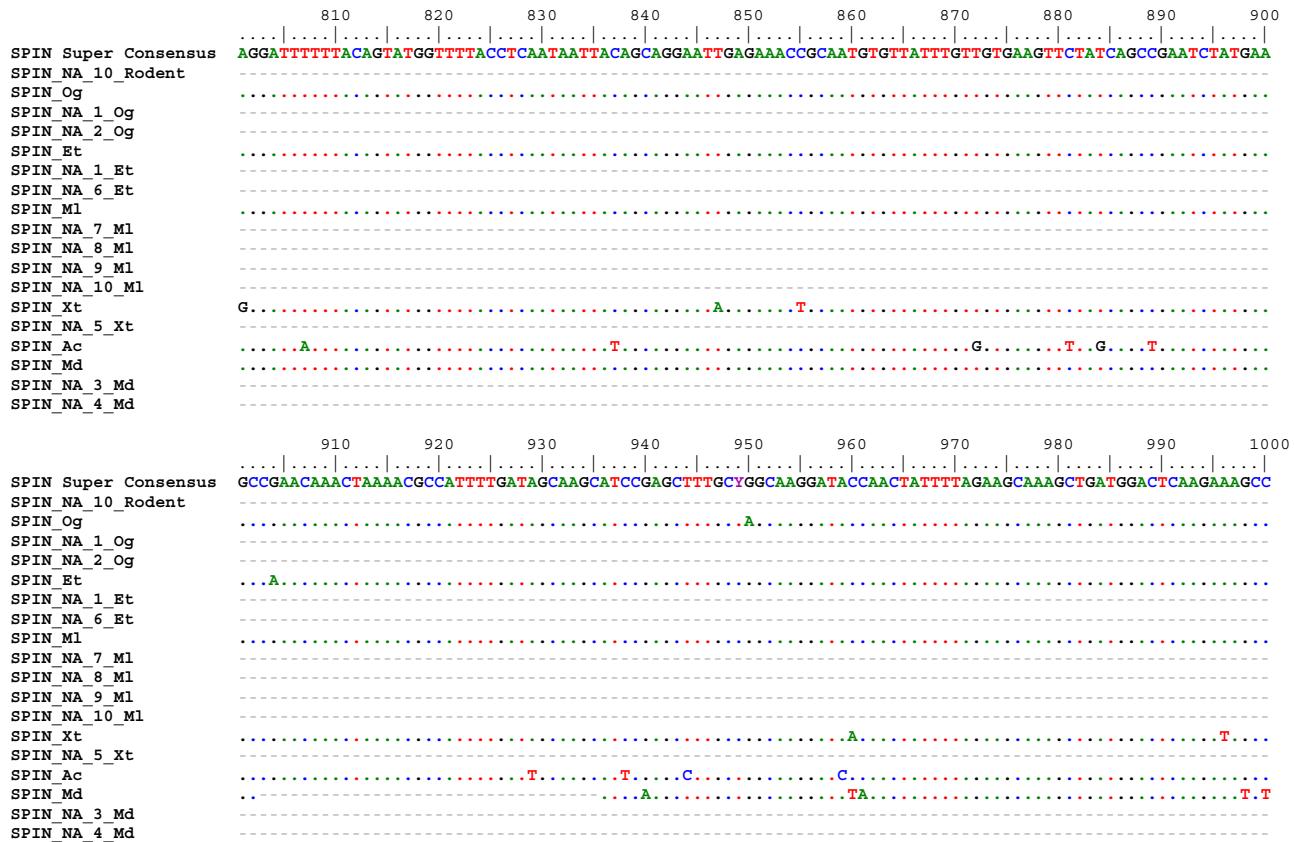


Fig. S1. (continued)



Fig. S1. (continued)

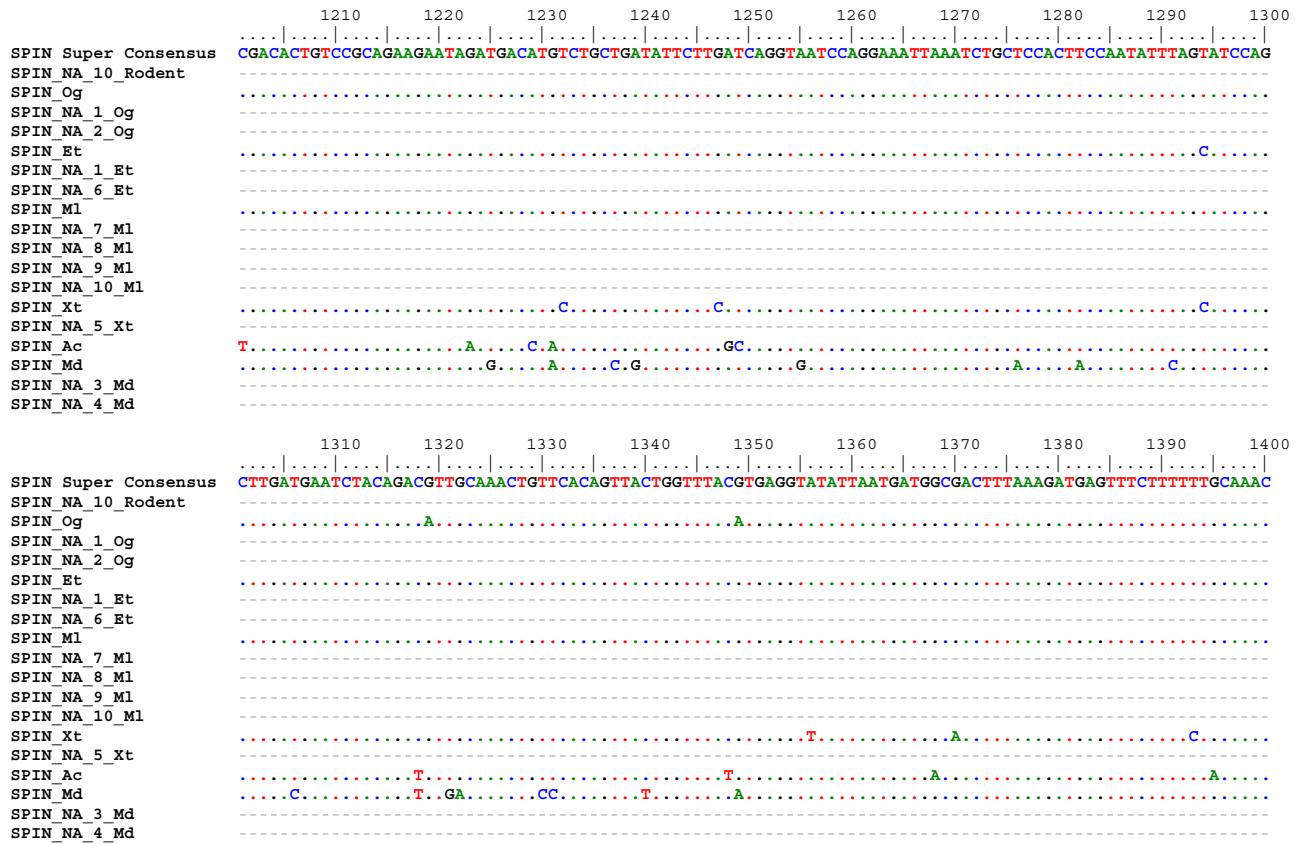


Fig. S1. (continued)

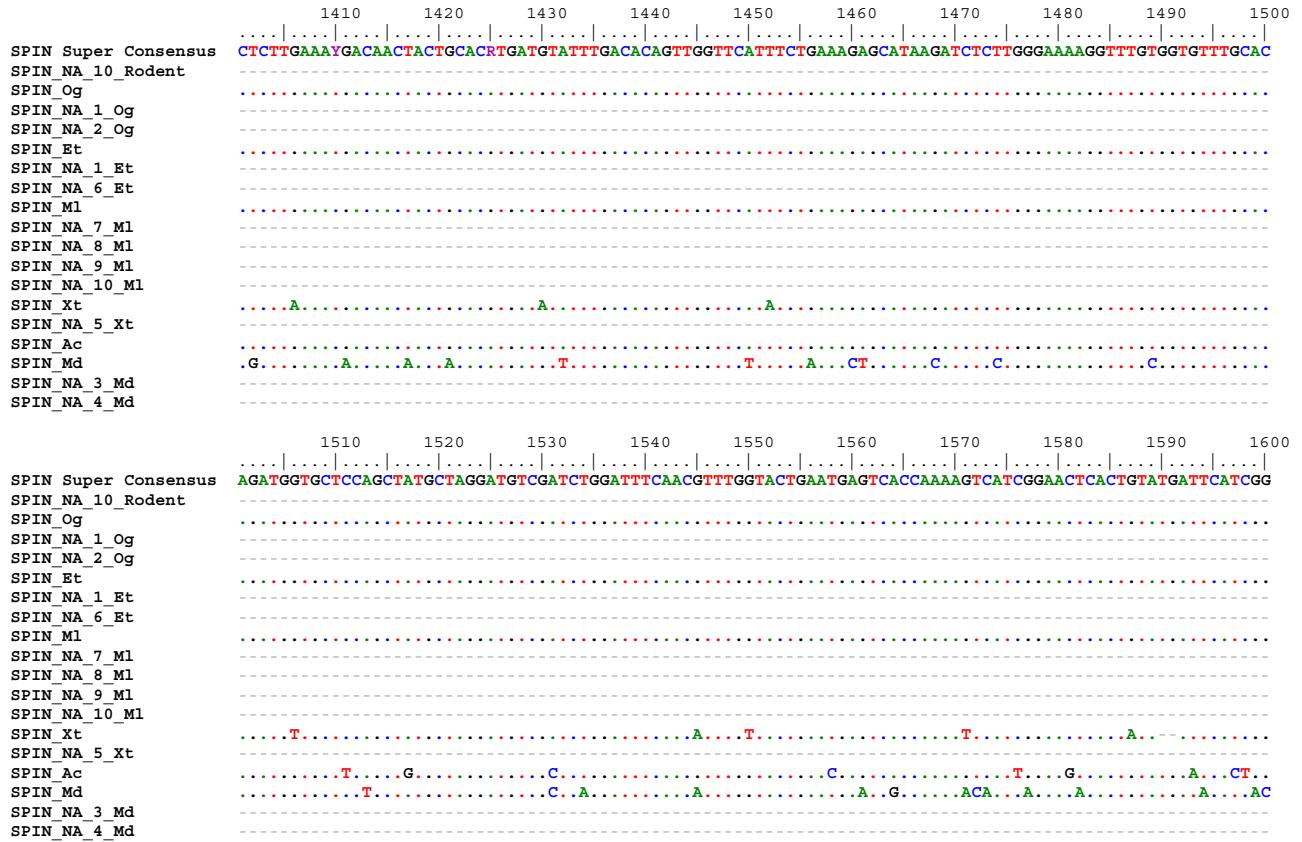


Fig. S1. (continued)

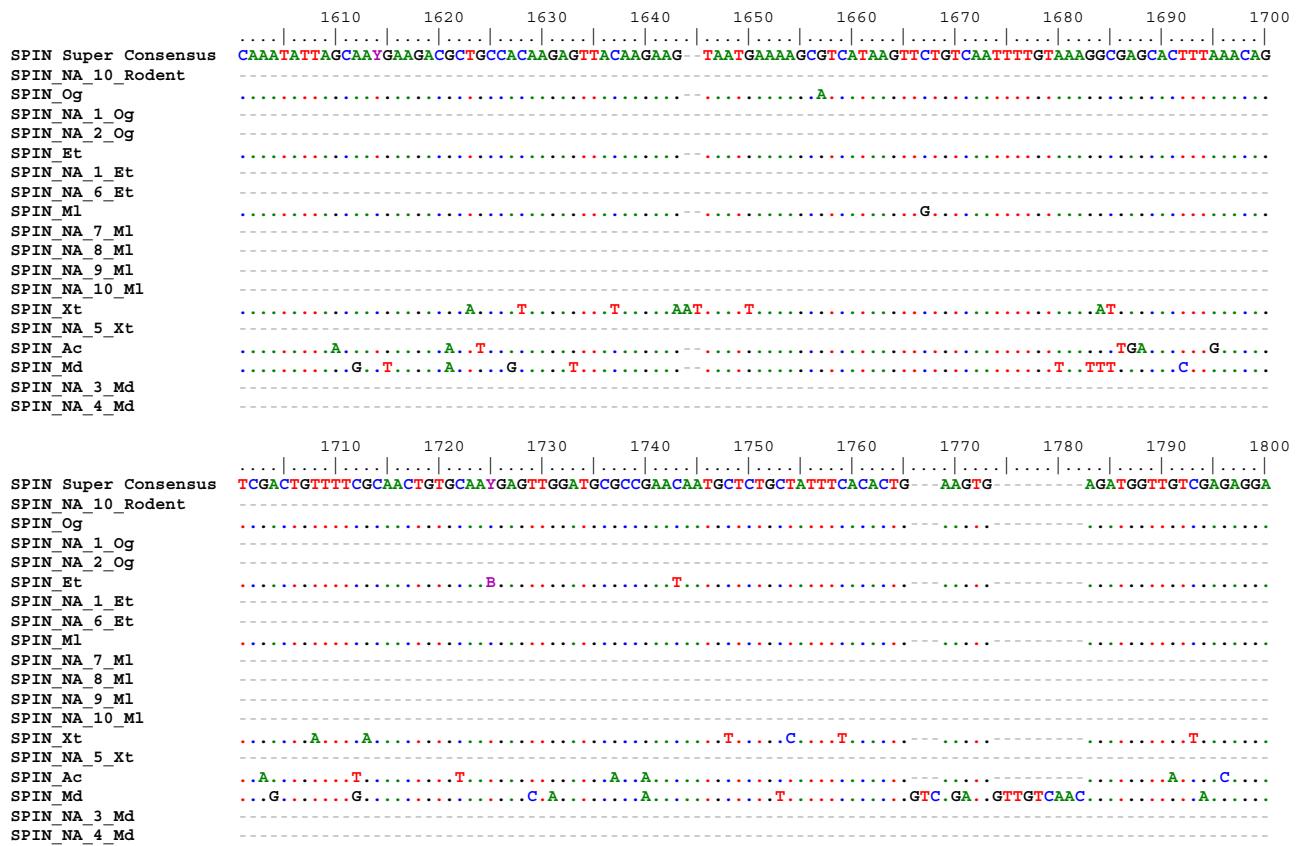
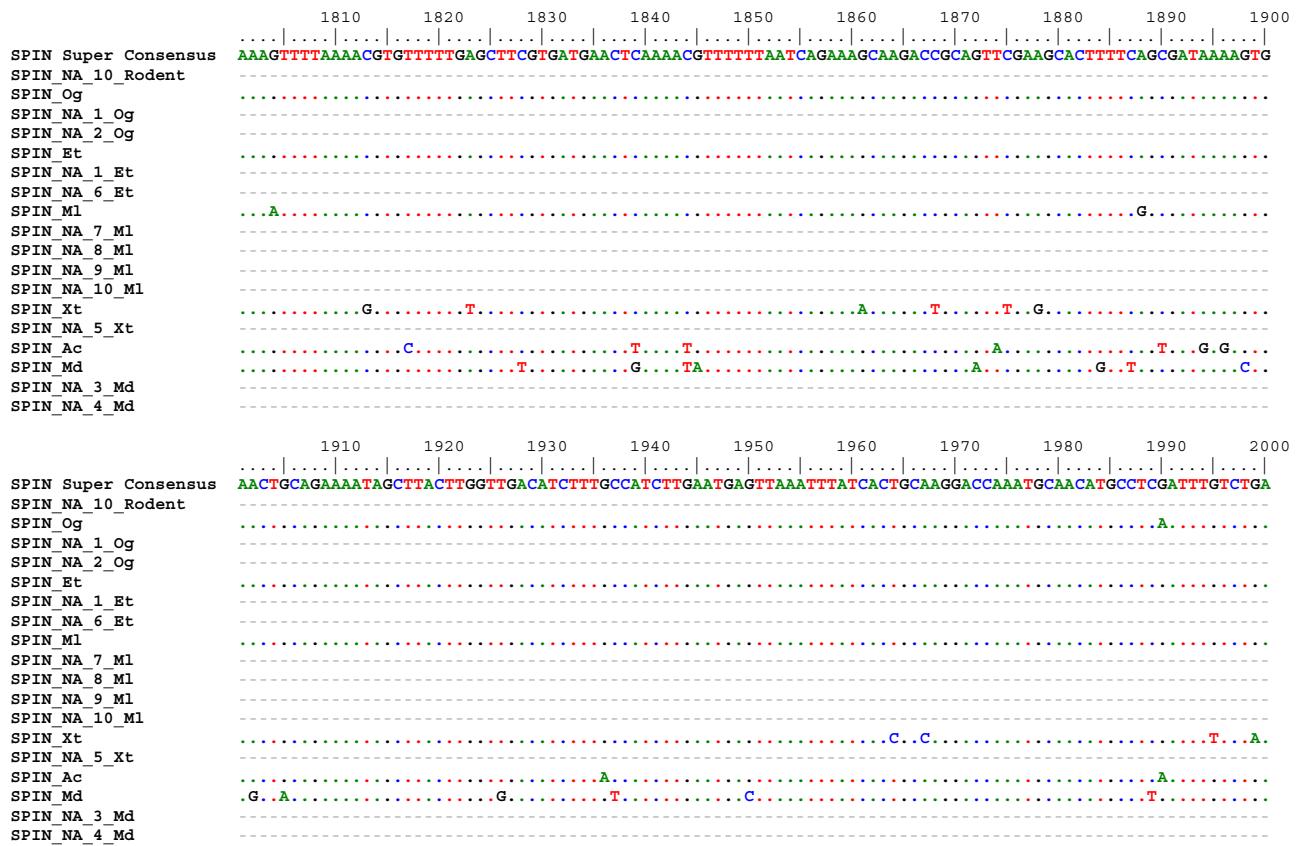


Fig. S1. (continued)



**Fig. S1.** (continued)

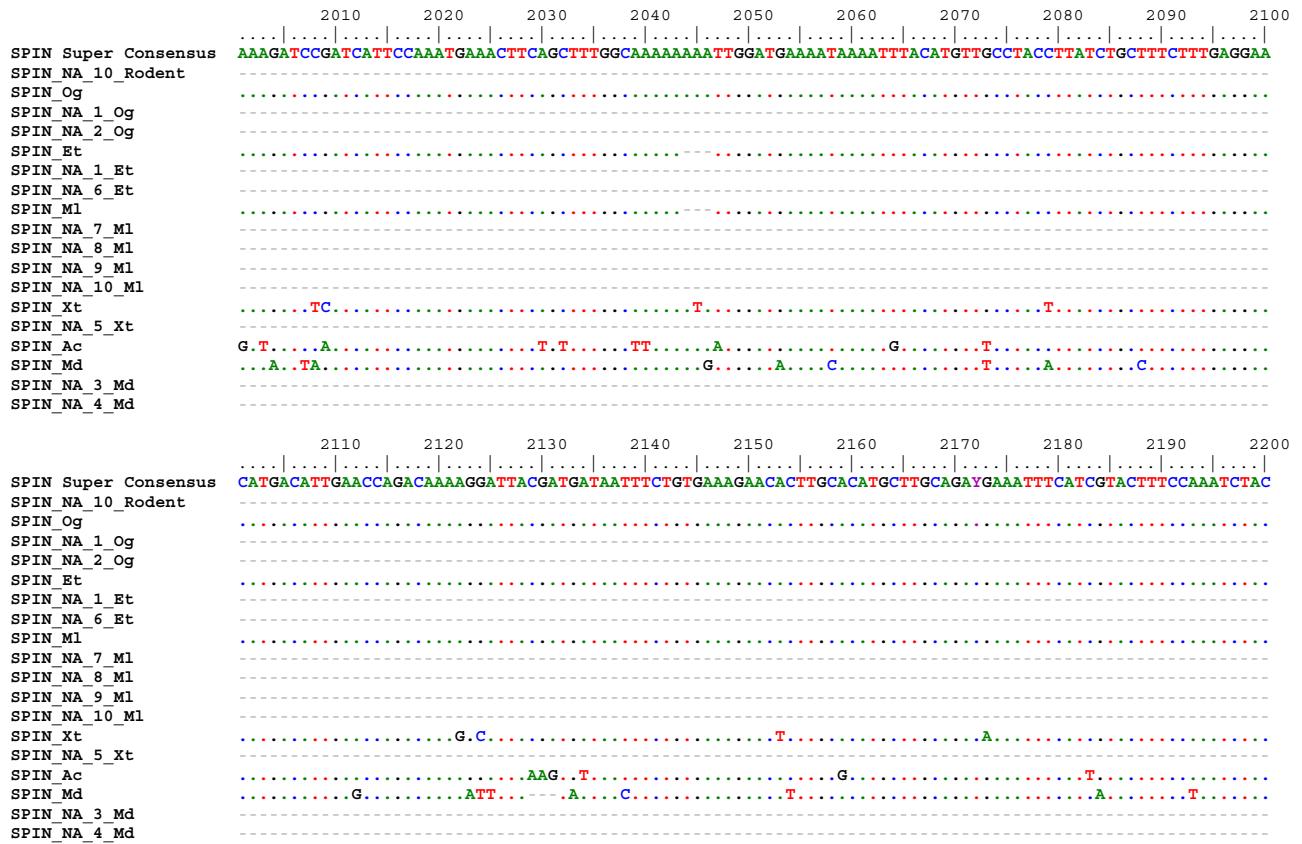


Fig. S1. (continued)

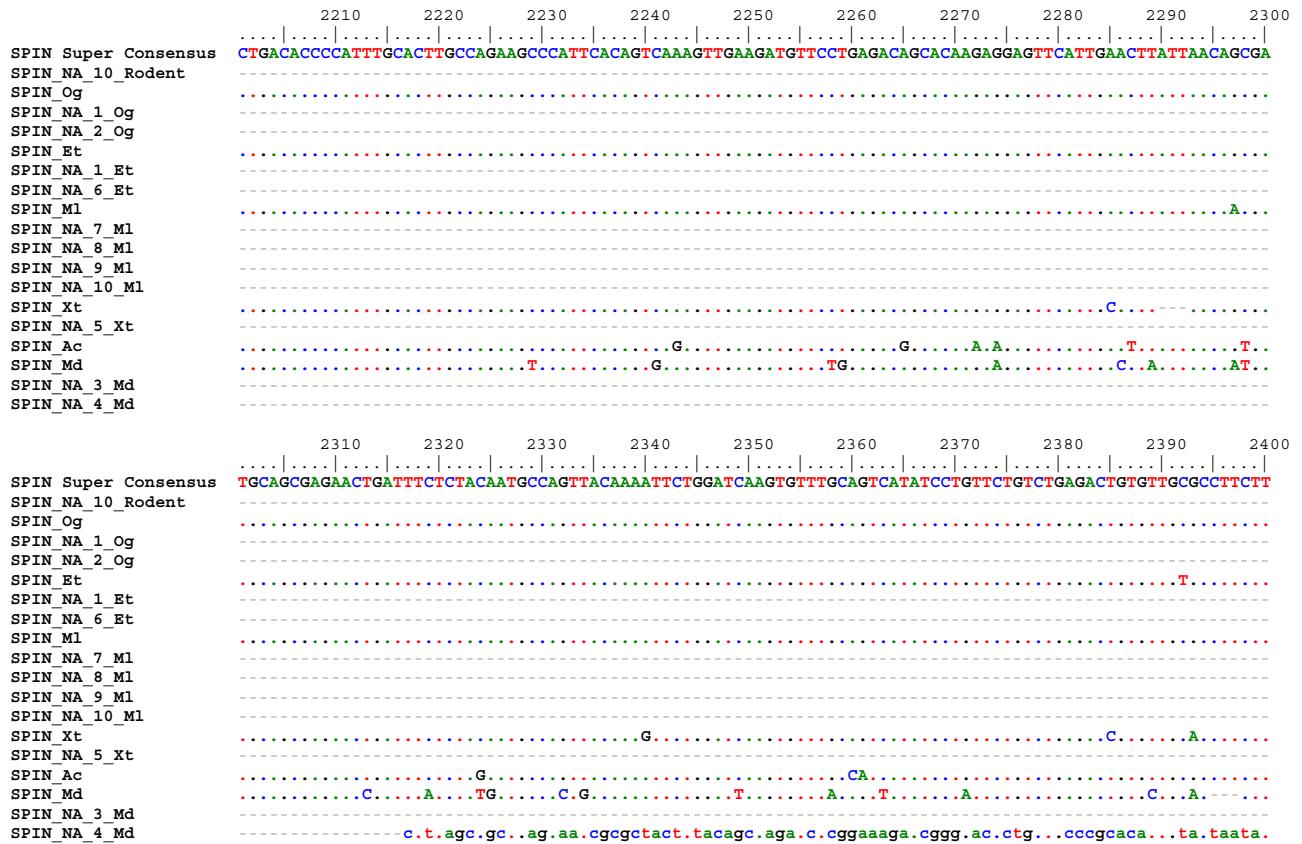


Fig. S1. (continued)

Sequence alignment showing variation from the SPIN Super Consensus across various SPIN NA strains. The top alignment covers positions 2410-2500, and the bottom alignment covers positions 2510-2600. Colored dots represent differences from the consensus sequence.

**Top Alignment (Positions 2410-2500):**

- SPIN Super Consensus: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC
- SPIN NA\_10\_Rodent: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC
- SPIN\_Og: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC
- SPIN NA\_1\_Og: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC
- SPIN NA\_2\_Og: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC
- SPIN\_Et: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC
- SPIN NA\_1\_Et: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC
- SPIN NA\_6\_Et: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC
- SPIN\_M1: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC
- SPIN NA\_7\_M1: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC
- SPIN NA\_8\_M1: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC
- SPIN NA\_9\_M1: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC
- SPIN NA\_10\_M1: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC
- SPIN\_Xt: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC
- SPIN NA\_5\_Xt: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC
- SPIN\_Ac: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC
- SPIN\_Md: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC
- SPIN NA\_3\_Md: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC
- SPIN NA\_4\_Md: CTTCCATTCCAACAAACATATCTTGTGAAACAGGGTTTCAGCTTGTTGGTTACAAGTCTAAATACAGAAGTAGACCTTGTTGGAAAGATGATCTTC

**Bottom Alignment (Positions 2510-2600):**

- SPIN Super Consensus: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC
- SPIN NA\_10\_Rodent: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC
- SPIN\_Og: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC
- SPIN NA\_1\_Og: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC
- SPIN NA\_2\_Og: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC
- SPIN\_Et: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC
- SPIN NA\_1\_Et: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC
- SPIN NA\_6\_Et: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC
- SPIN\_M1: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC
- SPIN NA\_7\_M1: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC
- SPIN NA\_8\_M1: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC
- SPIN NA\_9\_M1: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC
- SPIN NA\_10\_M1: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC
- SPIN\_Xt: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC
- SPIN NA\_5\_Xt: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC
- SPIN\_Ac: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC
- SPIN\_Md: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC
- SPIN NA\_3\_Md: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC
- SPIN NA\_4\_Md: GTGTTGCTCTTGCAAAAGACTGCCCGAGAAATTCTGATCTGGTGGAAAGAACATCTCAACCTTCNCACCTGACGTTGGCTTTACGCACTACTGTYGC

**Fig. S1.** (continued)

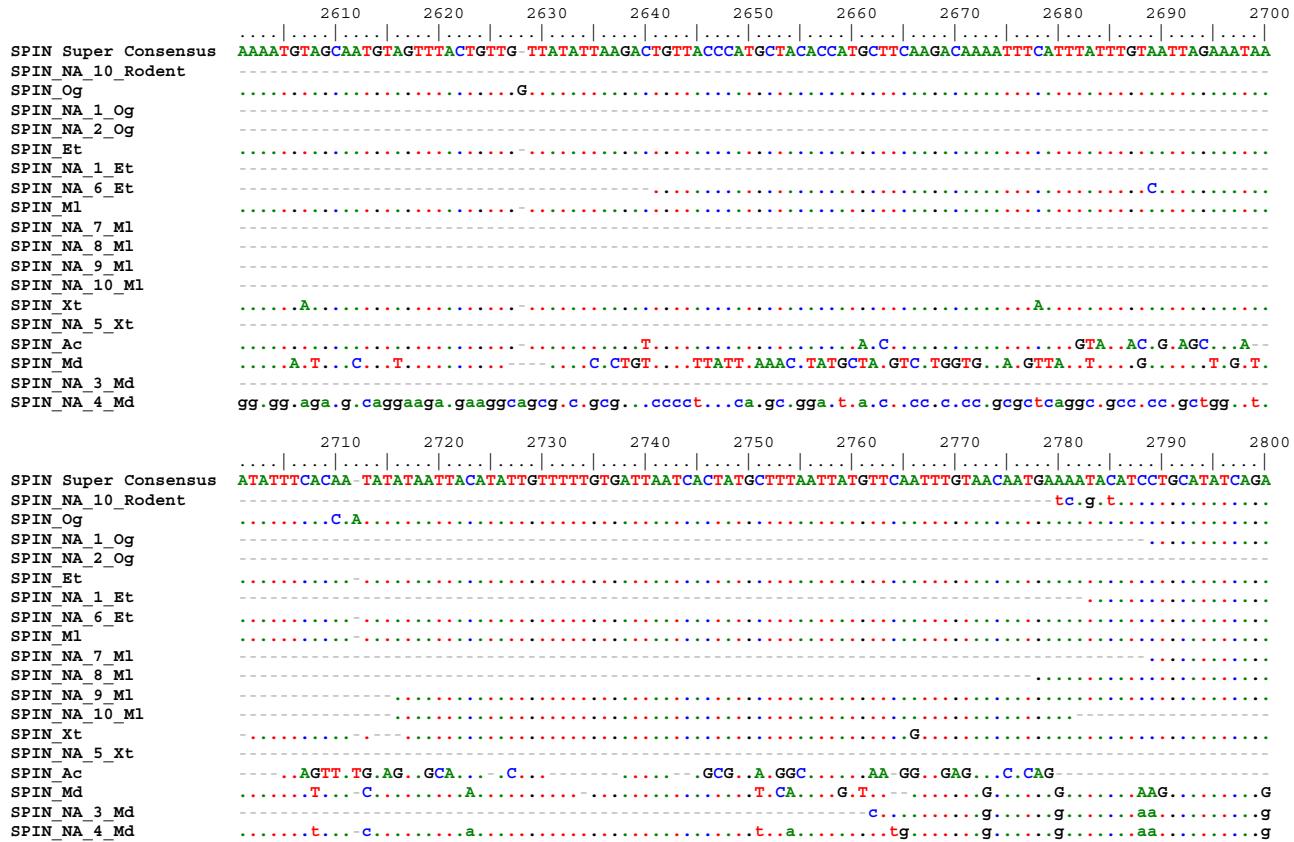


Fig. S1. (continued)



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**Fig. S1.** (continued)

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>SPIN\_NA\_1\_Og

**Fig. S1.** (continued)

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>SPIN\_Et  
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>SPIN\_M1  
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**Fig. S1.** (continued)

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**Fig. S1.** (continued)

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>SPIN\_NA\_11'\_Ac  
5' end  
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3' end  
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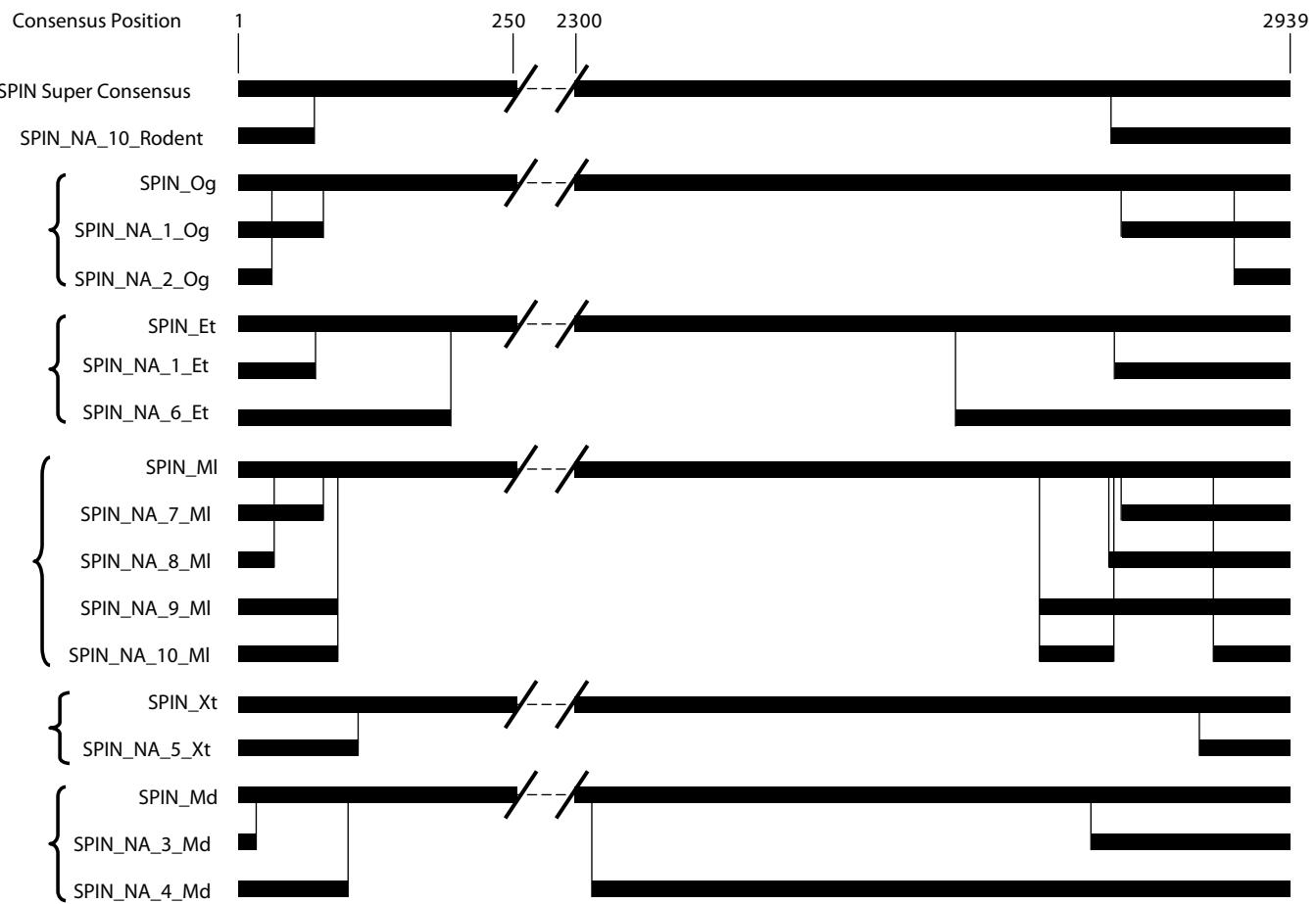
**Fig.S1.** (continued)

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actg

**Fig. S1.** (continued)



**Fig. S2.** Schematic of regions of shared identity between consensus sequences of full-length *SPIN* transposons and their derived MITEs in each species. MITEs are denoted by "NA" (for nonautonomous). The figure shows that the MITE families from different species have distinct deletion breakpoints with their cognate full-length *SPIN* transposon, indicating that each originated independently from a distinct deletion derivative. Species abbreviations: Rodent = mouse/rat, Og = *Otolemur garnettii* (bushbaby), Et = *Echinops telfairi* (tenrec), MI = *Myotis lucifugus* (bat), Xt = *Xenopus tropicalis* (frog), Ac = *Anolis carolinensis* (lizard), Md = *Monodelphis domestica* (opossum).

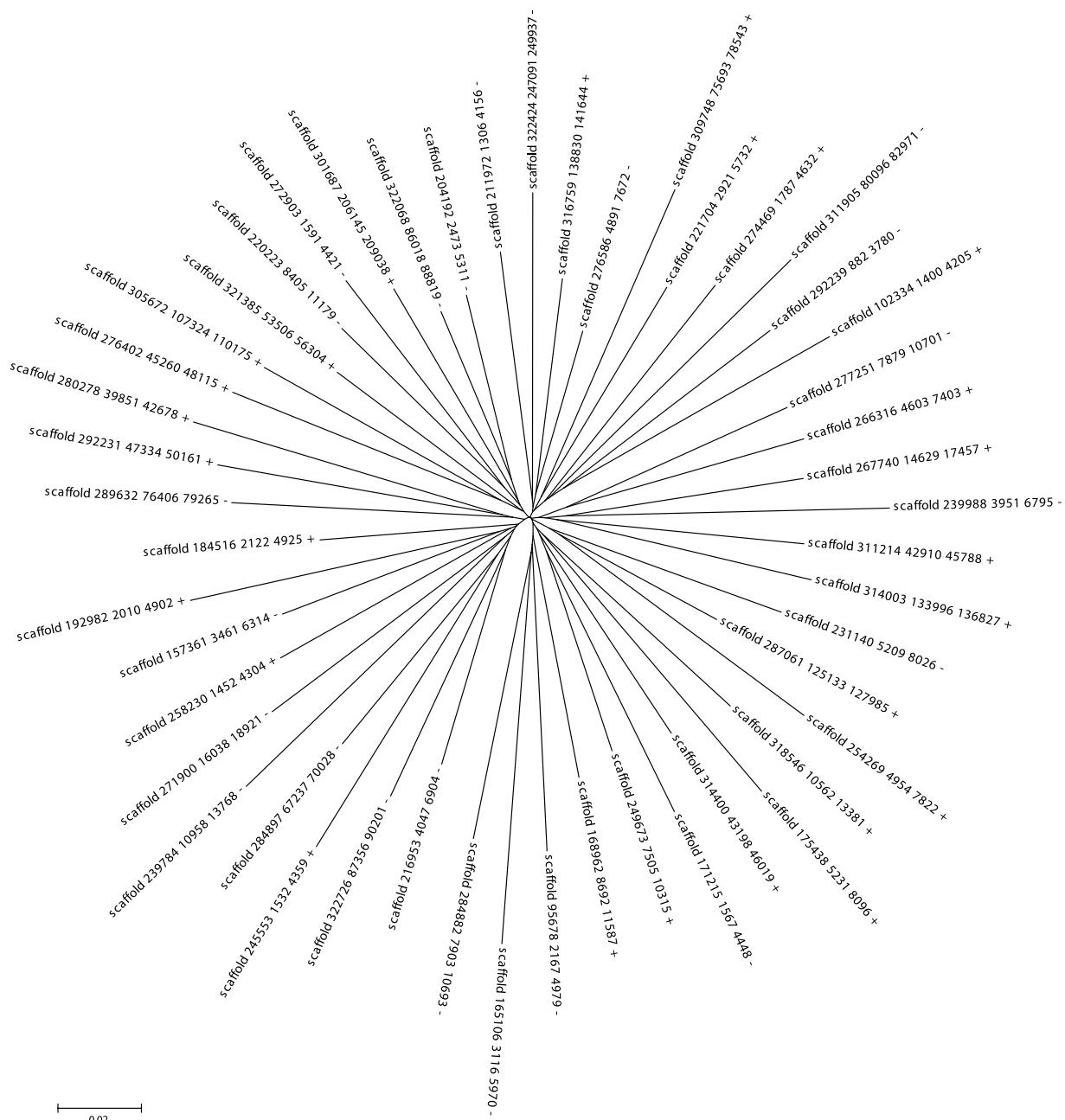
Empty Site #1  
 Tenrec - Position: scaffold\_96418:190-3546  
 Tenrec - TCTAGTCTCAGTCATAATTG-**CTTTATAT**CAGTGTTCT...ATAACCACGTAAAAATGCATTTGTTAATTCAAATTAA  
 Bat - tctagtgtcatacacacccgt**cttataat**-...-tcattt-ttgat--aaaaccg  
 Elephant - tctaattctcaatc---tc-**ctttatat**-...-tccttt-ttgatccaaatcca  
 Cat - tgttag-ctcacacacccgt**ttttaat**-...-tc-tttttgattcaaattcca

Empty Site #2  
 Bat - Position: gi|04559044gb|AAPE01636359.1:7114-10360  
 Bat - GATTAAATGATAAAATATAT**GTCTAGAA**CAGCGGTTCT...AGAAAACACTGGTCTAGAAGTTG--GG-CTCTGGGCTTG  
 Dog - ga-tcagtgtataaggcatat**gtcttga**-...-gtttaggactctgagctta  
 Cow - aa-ccagtgtataaaacatat**ttcttag**-...-gtaatataactctggactcg  
 Treeshrew - ga-ccagtgtgtaaaa--tat**gtcttga**-...-gttgtggattctggccag  
 Human - ga-ccagtgtataaaaccaa**gtcttga**-...-gttgtaagattctggactga  
 Elephant - ga-ccagtgtacaaaacatgc**atcttcaa**-...-attgtggactctggctgg  
 Armadillo - ga-ccagaaaataaaacatgt**gtcttga**-...-gttcaggactctggctga

Empty Site #3  
 Bushbaby - Position: scaffold\_89809.1-71393:45862-49069  
 Bushbaby - GGAAGAAAAGTCCTTGTC**CTTTAAC**actgtggttct...aaaactactg**ctttaacat**cactatggctccctctact  
 Human - tgaag---tcctttcttc**ctttaatg**-...-tcactatggctccctctagt  
 Dog - gaaagagaagcccccttcttc**ctttactg**-...-ttgcttggctccctgccc  
 Cat - gaaggagaagcccccttcttc**ctttaatg**-...-tcactctggctccctctgt

**Fig. S3.** *SPIN* orthologous “empty” sites. Multiple alignments showing the presence (top line, reference species) or absence of *SPIN* elements at orthologous loci in diverse mammalian species. In all cases, we found both TSD copies (underlined) and the individual *SPIN* insertion in the reference species but only one copy of the TSD and no additional sequences in the comparison species.

## A. *SPIN\_Et* in tenrec



**Fig. S4.** Phylogenetic relationship among members of the same *SPIN* family. Neighbor-joining trees of (A) a family of full-length elements from tenrec (*SPIN\_Et*), (B) a MITE family from bat (*SPIN\_NA\_9\_Mt*), and (C) a MITE family from bushbaby (*SPIN\_NA\_1\_Og*). For (A), all 49 full-length copies of *SPIN\_Et* were included, whereas for both (B) and (C), 50 copies of each family were randomly extracted from each genome and a multiple alignment was created by using ClustalX (v. 1.83). The neighbor-joining tree was constructed by using MEGA 3.1 (1,000 bootstrap replications). The star-like topology of each tree and the lack of subfamily structure are indicative of a single burst of transposition followed by a pattern of idiosyncratic evolution along each branch.

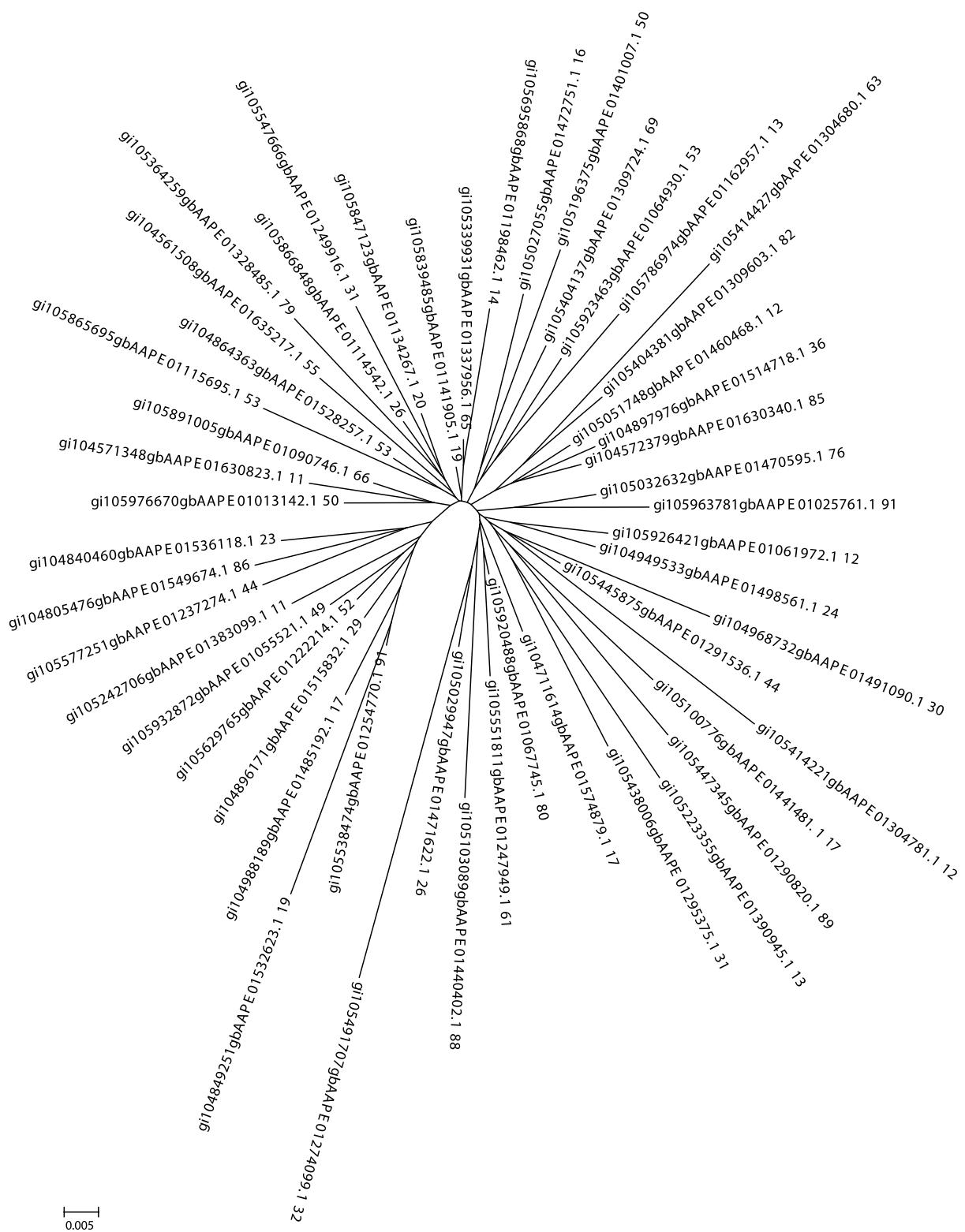
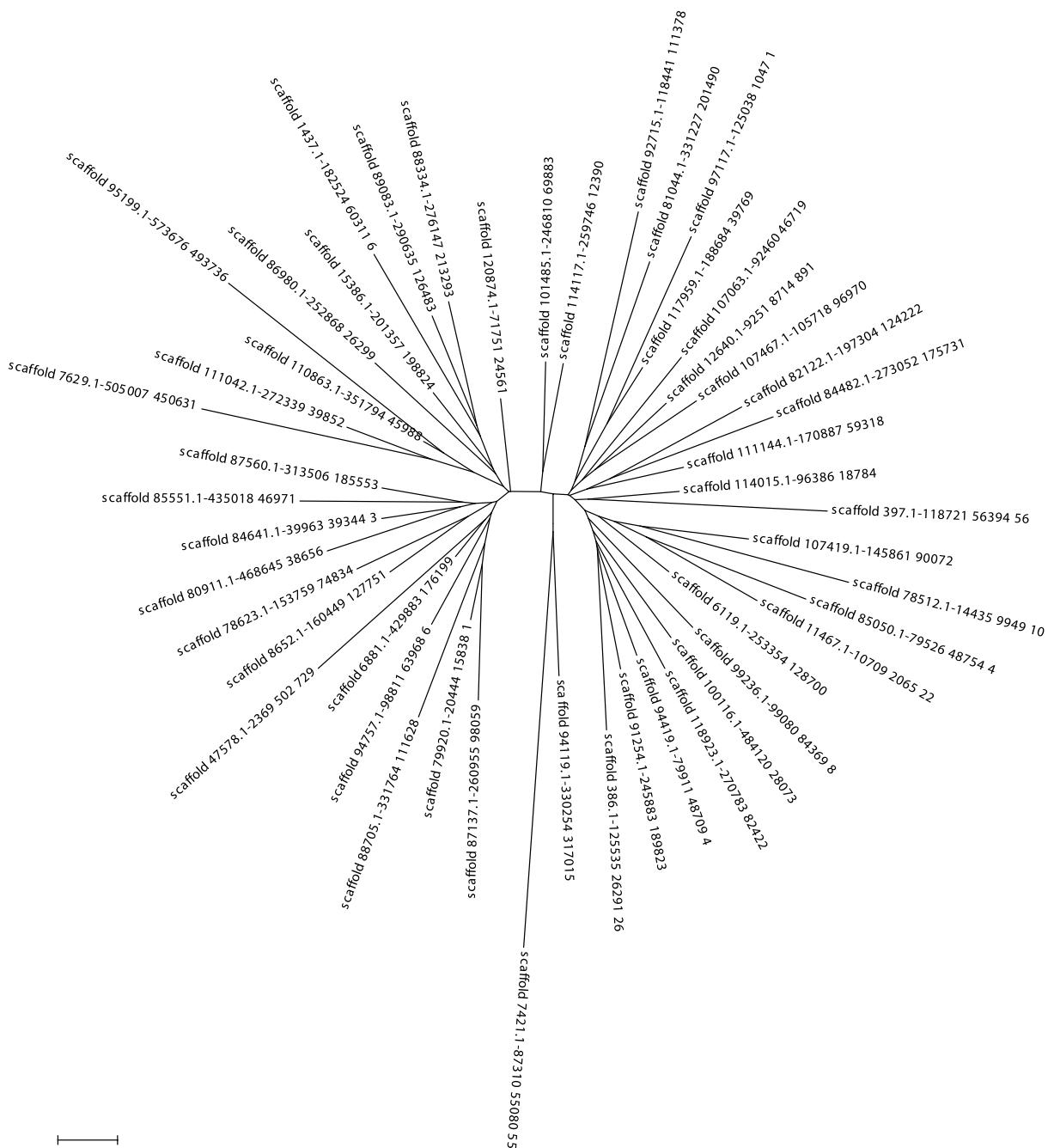
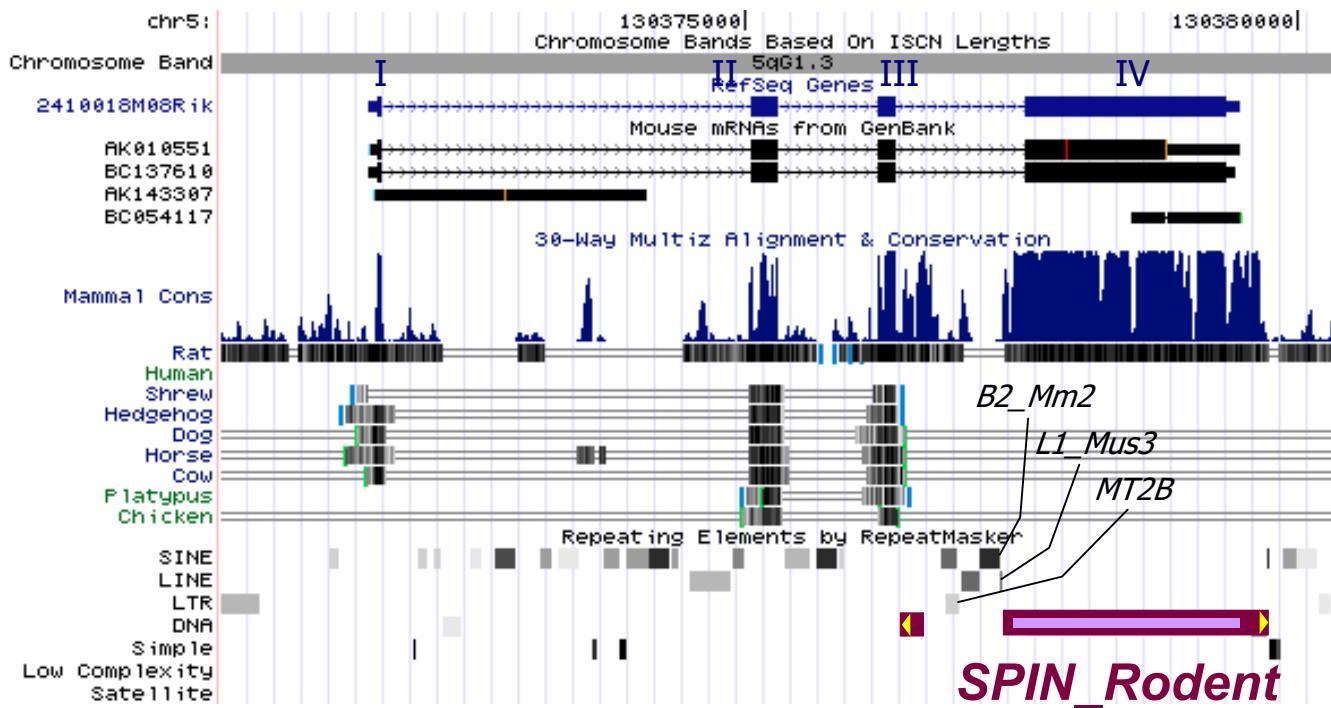
B. ***SPIN\_NA\_9\_MI*** in bat

Fig. S4. (continued)

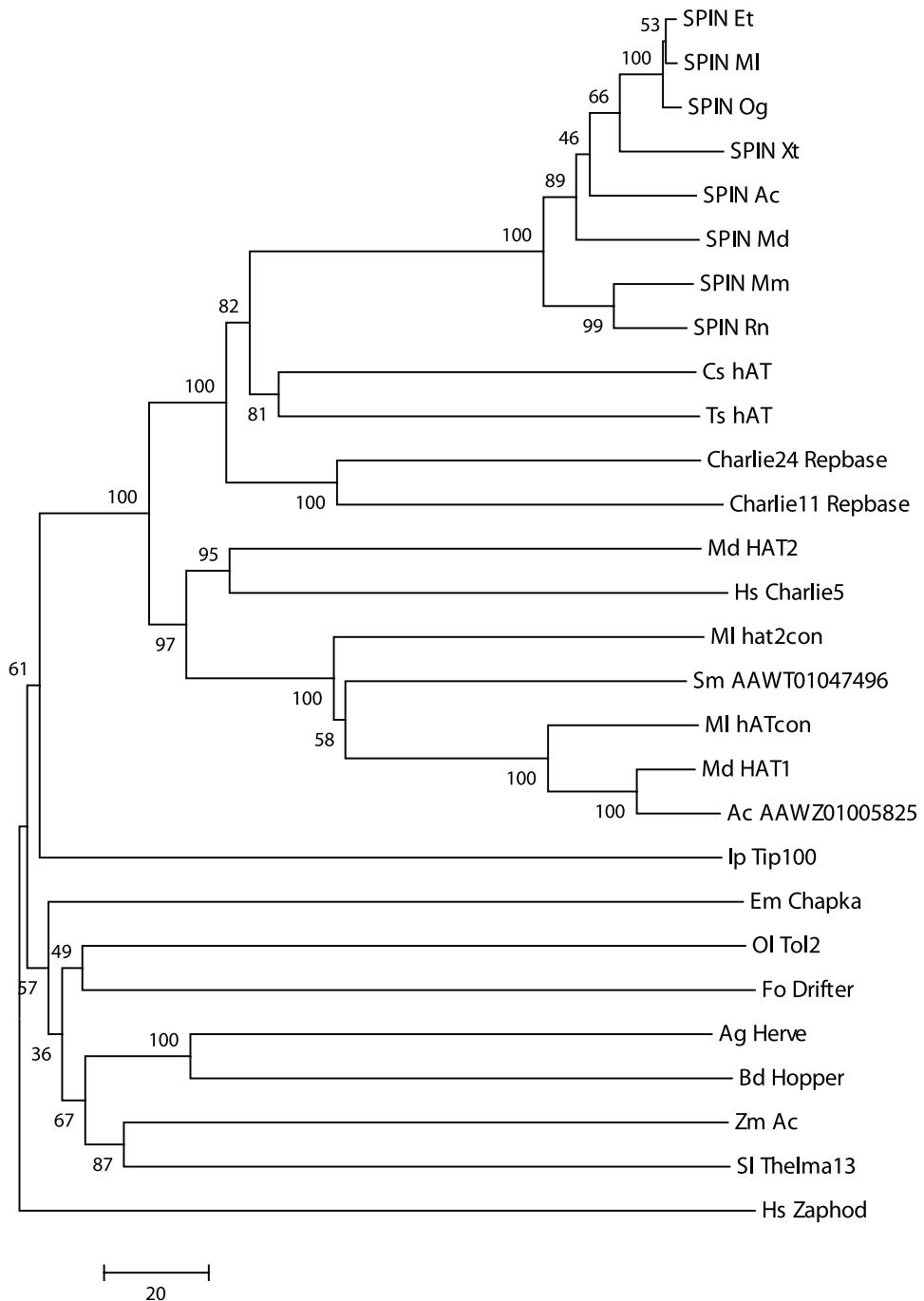
### C. ***SPIN\_NA\_1\_Og*** in bushbaby



**Fig. S4.** (continued)



**Fig. S5.** Murine-specific chimeric gene derived from *SPIN* transposase. This snapshot was extracted from the annotation of the mouse July 2007 (mm8) genome assembly implemented within the UCSC Genome Browser. The chromosomal region displayed is chr5:130,370,270–130,380,588. The dark blue rectangles at the top represent the four exons of RefSeq gene 2410018M08Rik (also known as *NM\_183088.2*). Exons I–III encode a CHCH domain (*pfam06747*), whereas exon IV is entirely derived from a *SPIN* transposase and, accordingly, is predicted to encode a *hATc* domain (*pfam05699*). The black rectangles depict corresponding exons identified in mouse cloned cDNAs deposited in GenBank. Below is the conservation track, which reflects the alignment of this mouse genomic region with other vertebrate genomes (species listed on the left). Exons I–III are present and conserved in most other mammals (but apparently not in human) and in chicken, whereas exon IV is absent from these species except rat, where all four exons are present and highly conserved. The last set of annotations at the bottom depicts the position of repeats given by Repeatmasker. The relative position of *SPIN\_Rodent* is shown by the purple bar, with the TIRs marking the boundaries of the element shown as yellow arrowheads, and the transposase ORF as a violet horizontal line. The Repeatmasker annotation shows that the 5' noncoding region of *SPIN\_Rodent* has suffered at least consecutive insertions of three other mobile elements (MT2B ERVL, L1\_Mus3 LINE, B2\_Mm2 SINE). These insertions did not disrupt the ORF, but likely caused the immobilization of the *SPIN\_Rodent* element.



### Species

Ac=Anolis carolinensis, Ag=Anopheles gambiae, Bd=Bactrocera dorsalis (fly),  
 Cs=Ciona savignyi (sea squirt), Em=Entamoeba moshkovskii, Fo=Fusarium oxysporum (fungus), Hs=Homo sapiens, Ip=Ipomoea purpurea (plant),  
 Md=Monodelphis domestica, Mi=Myotis lucifugus, Ol=Oryzias latipes, Sl=Silene latifolia (plant), Sm=Schmidtea mediterranea (planaria), Ts=Trichinella spiralis (roundworm), Xt=Xenopus tropicalis, Zm=Zea mays

**Fig. S6.** Phylogenetic relationship of SPIN transposase within the eukaryotic hAT superfamily. The neighbor-joining tree was constructed by using MEGA 3.1 (1,000 bootstrap replications) (2) based on a multiple alignment of the amino acid sequence of the SPIN transposases and various representatives of the hAT superfamily of transposases. Each sequence is identified by the species initials (abbreviations shown at the bottom of the tree), followed by the name of the corresponding transposon, if previously described. Other hAT elements newly identified in this study are designated by the suffix 'hAT'. The hAT transposases most closely related to SPIN are found in the chordate *Ciona savignyi* (hAT-Cs) and the roundworm *Trichinella spiralis* (hAT-Ts). However, each of these transposases has less than 45% amino acid similarity to SPIN.